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Dioecism in the trailing arbutus, with notes on the morphology of the seed

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The heteromorphic nature of the flowers of *Epigaea repens* has been the subject of occasional remark ever since the time of André Michaux. In his journal[11] (p. 138) written while exploring in the Alleghany Mountains in 1796, occurs this entry: "Le 2 Avril Epigea repens en pleine fleur comme les jours précedents: sur plusi. individus toutes les fleurs femelles sans rudiments d'Etamines et sur d'autres individus toutes les fleurs hermaphrodites." His Flora[12] (p. 250) contains this further note: "Flores omnes in nonnullis individuis abortivi."

In 1868, Thomas Meehan[10] presented a paper before the Philadelphia Academy of Sciences in which he called attention to the fact that the pistillate flowers have "fine cleft stigmas strongly recurved, exposing a glutinous surface; while the hermaphrodite ones keep the apex of the pistils closed." From this he inferred that the plant was practically dioecious; and an examination of a number of plants during the fruiting season apparently confirmed this belief, for he found that over half the plants set no fruit.

Asa Gray[4] published in 1876 a short article on the floral structure of *Epigaea*, in which he classifies the flowers in two main groups, each with two modifications. The first group is characterized by having well developed stigmas composed of five radiate lobes which are moist and glutinous and evidently well

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adapted for retaining pollen. This first group he subdivides on the basis of whether the stigma projects above the corolla or is included within the tube. In the " shorter styled form," however, the height of the stigma varies considerably, being " sometimes as low as the middle of the tube of the corolla, sometimes nearly up to the throat." This main group is further characterized by having aborted stamens. The stamens of this form show varying stages in abortion, from mere rudiments of filaments to a condition of fully developed filaments bearing anthers with occasional pollen grains. They are, however, always functionless and the form is evidently pistillate.

The second group is characterized by having small, erect, and comparatively smooth and dry stigmas which do not retain pollen readily. The ovaries in this form are apparently as well developed as those in flowers of the first group, and the styles show the same variations in length. This small stigma form has well developed stamens with anthers that abound in pollen. The stamens vary much less in length than do the pistils but there is " a tendency to having lower instead of higher anthers in connection with the short style." In conclusion Gray raises the question as to whether *Epigaea* is really dioecious or the small stigma form sometimes sets fruit; and suggests that the modifications in the length of the style point toward a heterostylous condition like that of the primroses.

Since Gray's time several papers dealing with the flowers of *Epigaea* have appeared. All of them agree in most particulars with his observations. Halsted[6] found all the kinds of flowers described by Gray, and notes that about one third of the flowers of the small stigma form have the stigmas even with the anthers. He measured the pollen grains from the longer and shorter stamens and found no difference in their size. As a difference in the size of the pollen of the different forms is common among heterostylous plants Halsted concludes that *Epigaea* shows no real evidence of heterostyly.

Wilson[15] examined a thousand plants, mostly from North Carolina. He found that the kind with perfect stigmas showed all gradations in length of style from the longest to the shortest. In only one of these large stigma plants did he find even aborted

anthers. In the kind with pollen-bearing stamens he found the same variations in length of pistil and an equal variation in length of stamen. The two organs do not show any correspondence in length, however, but all lengths of stamens are found associated with each length of pistil. Wilson concludes that the pistil in this form is functionless because of its imperfect stigma and that the species is really dioecious; and he makes the interesting observation that the flowers of the two forms differ, the corolla being from one third to one seventh smaller and generally more pink in the large stigma form than in the small stigma form. He also considers that *Epigaea* must have been trimorphic before becoming dioecious.

Miss Langdon[9] examined a large number of flowers and found all the types described above. She notes that several arrangements of anthers may occur with each length of stigma, and that while usually all the flowers of a cluster are of the same form occasional exceptions occur. For example, on one spray there was a cluster of medium-styled flowers and one of short-styled; and in one short-styled cluster was one long-styled flower. Bastin[1] in a paper on the structure of *Epigaea*, discusses the flowers but all his observations on this point are taken from Wilson's article.

The peculiar condition of the flowers of *Epigaea* was brought to the writer's attention, in connection with some work on heterostylous plants, by Gray's suggestion that *Epigaea* might be an example of a heterostylous species that either was approaching or had attained a dioecious condition. As will be noted from the papers cited above, there is considerable uncertainty as to whether *Epigaea repens* is really dioecious, that is, whether in the form having perfect stamens the pistil is incapable of producing seed; and as to whether there is any real evidence of a heterostylous condition. The writer has accordingly investigated these two points. In connection with this work, flowers from several localities in New England and New York have been examined, and a number of plants taken up and cultivated for experimental purposes. The writer wishes to acknowledge his indebtedness to friends who have sent him specimens and to Mr. F. V. Coville for generously sending information as to his methods of cultivating the plant.

EVIDENCE OF HETEROSTYLY

The claims as to heterostyly in *Epigaea* are based entirely upon the variation in the length of the pistils As Darwin[3] (p. 3) emphasized, however, in his original discussion of heterostylous plants, anatomical characters alone do not furnish conclusive evidence of heterostyly. The essential differences between the forms of a heterostylous species are physiological differences in the pistils and pollen. Unless it be proven that one form is wholly fertile only when pollinated with pollen from another form, we do not have conclusive evidence that the species is heterostyled.

In *Epigaea*, moreover, the anatomical evidence is anything but conclusive. Gray (loc. cit. 75), to be sure, divides the large stigma kind into two groups distinguished by different lengths of style, but there are all gradations from the longest style to the shortest (Wilson, loc. cit. 59). The small stigma form shows even less evidence of heterostyly, for both styles and stamens show all gradations in length and the stamens vary within much narrower limits than do the pistils (Gray, loc. cit. 75). And in place of the correlation of pistils of a given length with stamens of a different length characteristic of heterostylous flowers, we find in *Epigaea* any length of stamen associated with any length of pistil (Wilson, loc. cit. 59, and Miss Langdon, loc. cit. 11); and flowers with stigmas at the anther level are common (Halsted, loc. cit. 249). Moreover, as Halsted points out, the pollen grains from the stamens of different lengths show no difference in size; and this, while by no means conclusive, lessens the probability that the species is truly heterostylous. All these observations have been confirmed by the writer.

Experiments, however, show still more conclusively that *Epigaea* cannot be considered a heterostylous plant. The writer transplanted several wild plants with large stigma, each of which bore a dozen or more buds, and cultivated them out of doors. The plants apparently suffered no injury and all blossomed readily. While the pistils showed a wide variation in length they may be roughly classified as long-styled, mid-styled, and short-styled forms. Flowers of each of these forms were pollinated with pollen from long and mid-length stamens. No flower with stamens

corresponding in length to the shortest pistils has been found by the writer.

The pollen germinated readily, and sections made twenty-four hours after pollination showed the stigmas crowded with pollen tubes. The tubes, however, developed rather slowly, and it was only at the end of five days, 120 hours, that pollen tubes were found extending to the ovules, and in a few cases the polar nuclei appeared about to fuse with the male nucleus. It is noteworthy that the pollen from both lengths of stamens germinated with equal readiness on all lengths of pistil. Such would not be the case, of course, in a truly heterostylous plant.

DIOECISM

The evidence as to dioecism is much more satisfactory. Unquestionably the large stigma form is uniformly pistillate, for usually only mere rudiments of filaments appear and often none at all. Gray (loc. cit. 74), however, found in some anthers in a flower of this form a few "perhaps well formed pollen grains," but the anthers never opened. In the one thousand plants examined by him, Wilson (loc. cit. 59) found only one large stigma plant that showed even aborted anthers. The writer examined over two hundred plants of the large stigma form, and only three bore flowers with any rudiments of anthers. Microtome sections of these flowers were prepared, and while the four locules could in some cases be distinguished they contained merely masses of broken down cells.

The form with well developed stamens, on the other hand, has never been observed to set fruit; and as Gray (loc. cit. 76) points out, it is easy to ascertain in any case which kind of flower has matured fruit, because the style and stigma persist until the fruit is fully mature. Indeed, in the flowers in which the ovary has not developed, the style and stigma often persist for over a year, somewhat shrunken, to be sure, but still sufficiently well preserved to make it certain to which form the flower belongs. This unusual persistence of the style and stigma seems to be due to the fact that the carpellary bundles contain strongly lignified tracheids.

The pistil of this form, however, is, as Gray (loc. cit. 75) observed, apparently normal except for its smaller and rather

dry stigma. The writer experimented with the artificial pollination of this small stigma form both with plants cultivated in pots and with flowers kept in water. The pollen adheres to these stigmas to a considerable extent, though this is probably due to the fact that the wall of the pollen grain is somewhat mucilaginous, for the pollen adheres also to the style below the stigma and even to smooth objects, such as a glass rod. In no case, however, was a pollen grain observed to develop a tube when placed on a stigma of this kind. The pollen was retained without degeneration for some time, frequently for over a week, but it never established any organic connection with the pistil and could easily be brushed off. As these cultivated plants appeared perfectly healthy and afterward developed apparently normal new shoots, the failure of the pollen to germinate cannot be attributed to any unusual weakened condition of the pistils. Moreover, the same condition was found to occur in long-styled plants of the small stigma form growing under natural conditions. Although the stigma in these plants projected well above the stamens it was covered with pollen, probably as the result of the visits of insects. Not a single grain, however, had emitted a tube.

This evident sterility of the pistils in the staminate form seemed still more remarkable when microtome sections of the ovules were examined. At the time of fertilization the embryo sac of the large stigma form shows uniformly typical egg and synergids, well defined antipodal cells, and the two polar nuclei lying close together near the middle of the sac. In all probability it remains in this condition over winter (Coulter and Chamberlain,[2] p. 53). The ovules of the sterile small stigma form present an identical appearance both in size and in the condition of the embryo sac. It is of course possible that the nuclei of this functionless embryo sac have not undergone a reduction division. There appears to be no evidence, however, of a tendency toward apogamy, and these unfertilized ovules gradually degenerate.

We have then, in the small stigma form of *Epigaea*, flowers that are apparently perfect but functionally male. The morphological differences between the functional and non-functional pistils in this species are, as pointed out above, very slight and are confined to the stigmas. Their physiological differences are so

great, however, that pollen which germinates readily on the stigmas of one form is absolutely inert on those of the other, and *Epigaea repens* is functionally a dioecious plant.

A few other cases of plants that are apparently polygamodioecious but actually dioecious have been observed. Darwin (loc. cit. 288) noted that the "hermaphrodite" flowers of *Euonymus europaeus* are practically male, so this species is really dioecious. Mottier[13] (p. 377) and von Kirchner[8] (p. 116) have pointed out that the apparently polygamodioecious maples are functionally dioecious, because the anthers of the "perfect" flowers, although they contain some apparently normal pollen grains, never open.

Celastrus scandens is an apparently polygamodioecious plant, having pistillate and "perfect" flowers. Like the related *Euonymus europaeus*, however, it is dioecious in function, for the pistils of the "perfect" flowers are smaller than those of the pistillate and appear upon investigation to be non-functional. *Celastrus* is abundant in the vicinity of New Haven, Connecticut, and during the spring of 1911 the writer examined a large number of plants in this region. Particular care was taken to observe the plants bearing "perfect" flowers. Not only was there no indication of any development of the ovary but the flowers shriveled and dropped from the plant almost as soon as the pollen was shed. Microtome sections showed that the ovules in the pistillate flowers are more than ten times as large as those of the "perfect" flowers. Many of these non-functioning ovules contain, however, an apparently typical embryo sac.

The condition found in the maples is exactly the reverse of that found in *Epigaea* and *Celastrus*. In the latter species there are two kinds of flowers, one evidently pistillate, the other developing both ovules and pollen grains. In some species of maples, on the other hand, one kind of flower is evidently staminate and the other apparently perfect. In *Epigaea* and *Celastrus*, however, the ovules of the "perfect" flowers are never fertilized, and in the maples the anthers of the "perfect" flowers do not open. Notwithstanding their morphological differences, then, both types are functionally dioecious.

As Wilson (loc. cit. 59) states, there is in *Epigaea repens* an apparently constant difference in size between the large-stigma

and small-stigma flowers. If flowers of the two forms growing in the same locality are compared, the pistillate flowers seem to be uniformly smaller than the apparently perfect flowers. Darwin (loc. cit. 307) considers that this is generally the case in gynodioecious plants, and names a dozen such species in which the pistillate flowers are smaller than the staminate flowers. He notes also the fact that no such difference in the size of the corolla has been found in the two forms of androdioecious species.

The writer has been unable to demonstrate, however, any correlation of color of the corolla with the two forms of stigma. Wilson (loc. cit. 59) states that the corollas of the large stigma flowers are pink, while those of the small stigma flowers are white. This is apparently true in some localities; but in one lot of flowers sent from a locality in Maine nearly all the flowers of both forms were pink. Moreover, a small lot of flowers from New Hampshire showed the large stigma flowers small and white, while the small stigma flowers were larger and very deep pink.

Morphology of the seed

In the sections made for the purpose of determining the rate of growth of the pollen tubes several points were noted which seemed worthy of record, and the development of the seed has

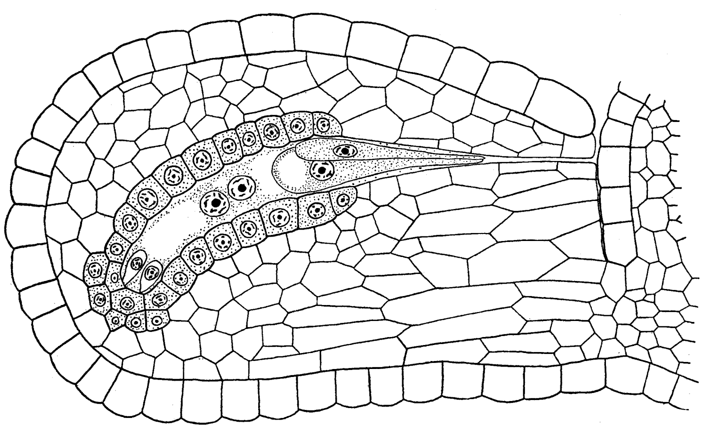


FIG. 1. Longitudinal section through ovule of small stigma form of *Epigaea repens* showing "tapetum" surrounding the lower portion of the embryo sac, elongated egg and synergid, saclike antipodal cells and polar nuclei. This ovule though non-functional is identical in appearance with the functional ones. ×400.

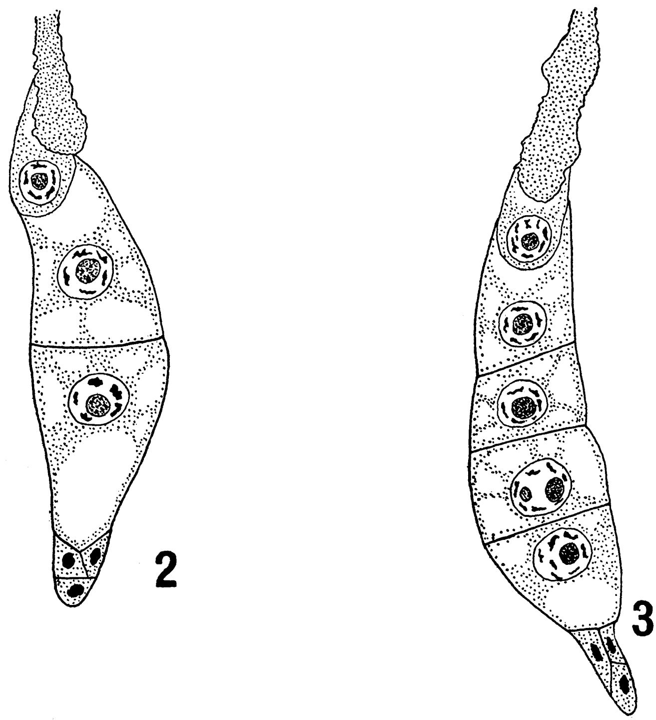
accordingly been traced. The mature embryo sac (FIG. 1) presents no unusual features except that it is somewhat curved and is prolonged, toward its micropylar end, into a narrow funnel-shaped region which contains the greater part of the egg and synergids. The nucellus either does not persist or else it coalesces so completely with the integument that they appear continuous.

The single massive integument, which makes up the bulk of the ovule, is differentiated into three rather definite regions: the epidermis, a middle storage region, and an inner tapetal region surrounding the embryo sac. The cells of the epidermis are large, regular, and rather thick-walled. The storage region shows little differentiation except that the cells in the funicular region are considerably elongated. The " tapetum " is composed of a single layer of cells except at the antipodal end, where it is usually two or more cells in thickness. Its cells have the large nuclei and dense granular cytoplasm characteristic of nutritive cells. It is noteworthy that the tapetum does not surround the narrow micropylar portion of the embryo sac, a fact which suggests that the micropylar portion may be a secondary development due to an encroachment of the growing egg and synergids upon the tissues of the integument.

As noted above, at the time of maturity of the embryo sac the polar nuclei lie close together in the middle of the sac. They remain entirely distinct, however, until fertilization occurs and the primary endosperm nucleus is formed by the simultaneous fusion of the polar nuclei with the male nucleus. The division of the primary endosperm nucleus is accompanied by the formation of a transverse wall dividing the embryo sac into two approximately equal chambers (FIG. 2). Each of these chambers is further divided by a cross wall resulting in a " four-chambered " embryo sac (FIG. 3). No division of the fertilized egg has occurred at this stage.

In the further development of the endosperm, cell divisions occur rapidly in all four of these " chambers." The cell divisions take place in various planes, and by the time the proembryo contains five or six cells it is impossible to distinguish the original cross walls which separated the four chambers of the embryo sac. There is thus no period of free nuclear division in the development of the endosperm in *Epigaea*.

Instances in which the first division of the primary endosperm nucleus is followed by the formation of a transverse wall across the embryo sac, are fairly common especially among the Sympetalae (Coulter and Chamberlain, loc. cit. 176); and the formation of a two-chambered embryo sac has been reported in the three subfamilies most closely related to the Ericoideae, that is,



FIGS. 2 and 3. "Chambered" embryo sac of *Epigaea repens*. ×600.
FIG. 2. Two-celled stage.
FIG. 3. Four-celled stage. The fertilized egg is partly covered by the remains of the pollen tube, and the antipodal cells have largely degenerated.

in the Pyroloideae, Monotropoideae, and Vaccinioideae (Hofmeister,[7] p. 141). The later development of the endosperm differs somewhat in the different groups. In the Vaccinioideae the endosperm is reported to develop only in the antipodal chamber while in the Pyroloideae and Monotropoideae endosperm is formed in both chambers. The only case in which, as in *Epigaea*, the embryo sac becomes divided into four superposed chambers all

of which take part in the development of the endosperm, is that of *Datura laevis* reported by Guignard[5] (p. 166).

A " four-chambered " embryo sac, formed, however, somewhat differently from those of *Datura* and *Epigaea*, is found in *Physostegia virginiana*. In this species Sharp[14] (p. 220) has recently observed that the division of the primary endosperm nucleus is accompanied by the formation of a longitudinal wall running through the middle of the embryo sac. The nuclei of the two resulting parts of the sac then divide and transverse walls are

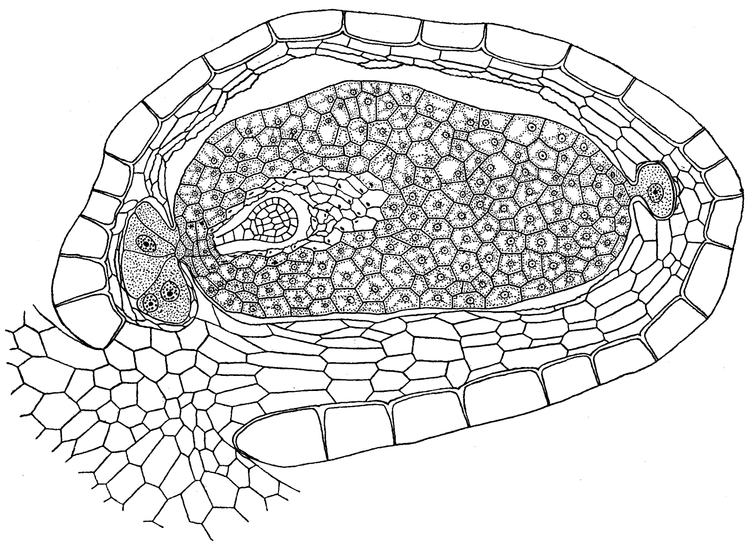


FIG. 4. Longitudinal section of developing ovule of *Epigaea repens*, showing haustoria which function for the nourishment of the endosperm. ×190.

formed. Further transverse divisions then give rise to a large-celled thin-walled tissue which fills the " endosperm lobe."

By the time the embryo of *Epigaea* reaches the quadrant stage the endosperm has a very considerable bulk and shows in longitudinal section fifty or more cells. At about this stage a knobshaped projection appears at either end of the endosperm. These outgrowths extend into the tissues of the integument and apparently function as haustoria to nourish the developing endosperm.

At first both haustoria consist of but a single large cell; and this is usually the condition of the antipodal haustorium at

maturity. In the formation of this cell a portion of one of the peripheral cells of the endosperm grows out into the tissues of the integument, increases greatly in size, and is later cut off by a cross wall. By the continual growth and division of the cell thus cut off, the micropylar haustorium develops rapidly until finally it consists of a group of eight or ten cells having dense granular contents and large nuclei. Frequently some of the cells of this haustorium contain two nuclei. The condition shown in FIG. 4 represents about the maximum development of the haustoria, and in the nearly mature seed they appear crushed and distorted among the tissues of the integument.

Haustoria of various kinds that function for the nourishment of the endosperm have been reported in numerous instances. Their occurrence and origin, together with the whole subject of the nourishment of the developing embryo sac, have been discussed by Coulter and Chamberlain (loc. cit. 104–113). They mention haustoria of various forms, developed from the micropylar and chalazal ends and even from the sides of the sac. Sometimes the haustoria develop from protrusions of the sac itself; but in other forms the synergids, the antipodal cells, the suspensor, and one of the row of megaspores have been reported to take part in the formation of haustoria. The structures found in *Epigaea* are noteworthy as rather simple haustoria which originate directly from the endosperm at a comparatively late stage in its development.

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